USING THE NLIN PROCEDURE TO COMBINE ESTIMATES OF RELATIVE POTENCY

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Abstract

The estimation of relative potency from indirect biological assays, assuming parallel line model has been thoroughly discussed by Finney(1978). Finney has also given a method for combining estimates of relative potency when the same test preparation is evaluated over s (s>1) assays. Finney's formula is not applicable when pooling estimates from k (k>1) replicates of the same test preparation from a single assay. In this case, the replicates are correlated due to a common standard. The current paper discusses two methods for finding the appropriate weights for the pooled estimate of relative potency in the case of correlated estimates. The first method uses the formulae for approximate standard errors of functions of random variables (Stuart & Ord, 1987) to obtain the covariance matrix, while the second method looks at a non-linear reparameterization of the model. A numerical example is also given using the NLIN procedure to produce all necessary parameter estimates.

1. Introduction

The estimation of relative potency from indirect biological assays, assuming a parallel line model has been thoroughly discussed by Finney(1978). The experimental design involves c (c>1) unknown test preparations, whose potency is to be compared against a known standard preparation. Each preparation is tested at d (d>2) specified doses, applied to n (n>1) subjects. The choice of doses depends on knowledge of the dose-response relation, ideally limited to the linear region. The estimation procedure given by Finney permits tests on the modelling assumptions, as well as biological considerations implicit in the conditions of similarity.

Finney has also given a method for combining estimates of relative potency when the same test preparation is evaluated over k (k>1) assays. The estimates of the relative potency from each of the k assays are pooled together to give a weighted estimate, $\bar{m}$, with the weights equal to the inverse of the variance. The expression is defined by

$$\bar{m} = \sum_{i=1}^{k} (m_i / \mathrm{Var}(m_i)) \; / \; \sum_{i=1}^{k} 1/\mathrm{Var}(m_i) \qquad (1.1)$$

where m_i = log relative potency estimate from the i-th assay, i=1,2,...k,
$\mathrm{Var}(m_i)$ = variance of m_i.

Tests for evaluating homogeneity of the relative potency estimates and variances are also given.

Formula 1.1 is not applicable when pooling estimates from k (k>1) replicates of the same test preparation from a single assay. In this case, the replicates are correlated due to a common standard. In the following sections, two methods are discussed for finding the appropriate weights for calculating the pooled estimate of relative potency in the case of correlated estimates. A straight-forward generalization of formula 1.1 is given for calculating the pooled estimate of relative potency. A numerical example is also given using the NLIN procedure to produce all necessary parameter estimates.

2. Estimation of relative potency from k replicates of a test preparation

Suppose k replicates $T_1, T_2, \ldots T_k$ of the same test preparation are assayed against a standard preparation, S. The relative potency of the test preparation can be estimated from the model given below, in which k+2 parameters are estimated from a single parallel line model.

Model:

$$y_{sjl} = \alpha_s + \beta x_j + \varepsilon_{sjl} \quad \text{for the standard, and}$$
$$y_{ijl} = \alpha_i + \beta x_j + \varepsilon_{ijl} \quad \text{for replicate } T_i,\ i=1,2, \ldots k, \qquad (2.1)$$

where

y_{sj} = the response of the l-th subject with the j-th dose, using the standard preparation,

y_{ij} = the response of the l-th subject with the j-th dose, from the i-th replicate of the test preparation,

x_j = j-th dose metameter,
= log (z_j) where z_j is the j-th dose,

α_s = intercept for the standard preparation,

α_i = intercept for i-th replicate, i=1,2, ...k,

β = common slope,

ε_{sj}, ε_{ij} = random error terms with the usual assumptions of independence, homogeneity and normality.

This model gives k estimates of log relative potency of the test preparation, which can be pooled to obtain a single point value. If a_s, a_i, and b are the estimates of α_s, α_i, and β respectively, then the estimated log relative potency of the i-th replicate is given by

$$m_i = (a_i - a_s)/b \qquad i=1,2,....,k. \qquad (2.2)$$

Due to the correlation between the estimates a_i (i=1,2,...,k), a_s, and b, the estimates m_1, m_2, ..., m_k are also correlated. Suppose that the estimated covariance matrix of m_1, m_2, ..., m_k is given by W. Then the estimates m_1, m_2, ..., m_k can be pooled to give a weighted estimate of the log relative potency, namely,

$$\bar{m} = (J^tW^{-1}J)^{-1} J^tW^{-1} (m_1,m_2,...,m_k)^t \qquad (2.3)$$

with

$$Var(\bar{m}) = (J^tW^{-1}J)^{-1} \qquad (2.4)$$

where J is a kx1 vector of unities, the superscript t denotes the transpose of a matrix, and $(m_1,m_2,...,m_k)$ is a 1xk vector of the estimates of log relative potency of the k replicates. When the estimates are uncorrelated, as in the case of estimates obtained from several independent assays, expression (2.3) reduces to (1.1).

3. Using the NLIN procedure to obtain the estimated covariance matrix of the correlated estimates of log relative potencies

Assuming model (2.1), the estimated covariance matrix of the estimates of log relative potency can be obtained by either one of the following two methods.

i. Method 1 - Estimating the covariance matrix of non-linear functions of random variables

Following Stuart and Ord (1987), we approximate the estimate of the standard errors using a Taylor Series expansion of a function of random variables. Let $\theta = (\alpha_s,\alpha_1,......,\alpha_k,\beta)^t$ be the (k+2)x1 vector of regression coefficients in model (2.1). Then

$$\mu = H(\theta) = ((\alpha_1-\alpha_s)/\beta, ..., (\alpha_k-\alpha_s)/\beta)^t$$
$$= (h_1(\theta),........, h_k(\theta))^t$$

is the kx1 vector of log relative potencies of replicates T_1,.....T_k. Let $H'(\theta)$ be the kx(k+1) matrix of partial first derivatives of $H(\theta)$,

$$H'(\theta) = (\delta h_1(\theta)/\delta\theta_i,......, \delta h_k(\theta)/\delta\theta_i)^t.$$

If $\hat{\theta}$ and $\hat{\mu}=H(\hat{\theta})$ are estimates of θ and μ respectively, then the covariance matrix of $\hat{\mu}$ is approximately equal to

$$Var(\hat{\mu}) = \hat{\sigma}^2 H'(\hat{\theta}) S (H'(\hat{\theta}))^t \qquad (3.1)$$

where $\hat{\sigma}^2$ S= covariance matrix of $\hat{\theta}$. The proof can be found in Stuart & Ord (1987).

If a_s, a_1, a_2,...., a_k, and b are the least squares estimates of α_s, α_1, α_2, ...,α_k, and β respectively, then $\hat{\theta} = (a_s, a_1, a_2,...., a_k, b)^t$ and $\hat{\mu} = (m_1, m_2,....,m_k)^t$. Carrying out the matrix multiplication given in (3.1), we get the approximate formula for the variance of m_i to be

$$w_{ii} = Var(m_i)$$
$$= (v_{ii} - 2m_i v_{ib} + m_i^2 v_{bb})/b^2 \qquad (3.2)$$

where v_{ii} =variance of $(a_i - a_s)$, v_{ib} = covariance $((a_i - a_s), b)$, and v_{bb} = variance of b. The approximate formula for the covariance of m_i and m_j $i \neq j$ is

$$w_{ij} = Cov(m_i, m_j)$$
$$= (v_{ij} - m_i v_{jb} - m_j v_{ib} + m_i m_j v_{bb})/b^2 \qquad (3.3)$$

where v_{ij} = covariance $((a_i - a_s), (a_j - a_s))$.

The covariance matrix of b, a_s, and a_i, i=1,2,...k can be obtained using the GLM procedure, and simple manipulation of the matrix using the IML procedure yields the covariance matrix of the estimates of log relative potency. Further calculations to produce the pooled estimate as given in formula 2.2 can easily be carried out using the IML procedure code.

ii. Method 2 - Non-linear reparameterization of model 2.1

Since the true log relative potency of replicate T_i is given by $M_i = (\alpha_i - \alpha_s)/\beta$, we can rewrite the model (2.1) as

$$y_{sj} = \alpha_s + \beta x_j + \varepsilon_{sj} \text{ for the standard,}$$

and (3.4)

$$y_{ij} = \alpha_s + \beta x_j + \beta M_i + \varepsilon_{ij} \text{ for replicate } T_i, i=1,2, ...k,$$

with parameters α_s, β, M_i, i=1,2,.....k. The normal equations for the above non-linear model are equivalent to the normal equations from the linear model (2.2). Hence, the solution to the normal equations (or the estimates of α_s, β, M_1,..., M_k) are identical to those obtained from the linear model (2.2). The NLIN procedure can be used to obtain the estimates as well as the covariance matrix of the estimates m_1,..., m_k directly without further manipulations of the matrices as required in method 1. Computationally, this is an easier approach than Method 1 since all the necessary estimates are obtained in a single step.

4. Numerical Example

Three replicates T_1, T_2, and T_3 of a test preparation T were assayed against a known standard preparation S. All preparations were assayed at doses 0.025u/ml, 0.50u/ml, and 0.10u/ml corresponding to previously determined linear region of the dose-response relationship. The sample size used was 10 animals per each dose-replicate (or standard) combination. In addition 10 animals were observed as the vehicle control group, yielding a total of 130 animals in the study.

Prior to performing regression analysis, descriptive measures for the data were obtained and seven animals were excluded from further analysis due to the following reasons: i. Six animals were excluded owing to biological considerations; ii. One animal was found to be a statistical outlier based on Dixon's test.

Method 1: Linear Modelling

As a first step in the regression analysis, the GLM procedure was used to test for the parallel line model (common slope). The interaction term of the model

RESPONSE = TEST LDOSE LDOSE*TEST

where LDOSE = Log (Base 2) of Dose and TEST is a CLASS variable with levels S, T_1, T_2, and T_3, was found to be not significant at a 10% level, indicating the appropriateness of the parallel line model. Next, model (2.1) was fit to the data using the REG procedure and the parameters and the estimated covariance matrix of a_1, a_2, a_3, a_s, and b were obtained using the commands OUTEST and COVOUT. The IML procedure code was utilized to calculate the variance-covariance terms as per formulae (3.2) and (3.3). The estimated slope of the parallel line model was found to be 0.842 and the estimates of log (to the base 2) of the relative potencies of T_1, T_2, and T_3 were -0.0627, -0.1516, and 0.197 respectively. The covariance matrix was estimated to be

	T_1	T_2	T_3
T_1	0.0193	0.0093	0.0093
T_2	0.0093	0.0194	0.0092
T_3	0.0093	0.0092	0.0185

The results from the linear models are given in Appendix A. The three estimates of log relative potency were combined using formula (2.3) and the IML procedure. The pooled estimate of log relative potency was found to be .00050 with an estimated variance of 0.0125.

Method 2: Non-linear reparameterization of the model

Using the NLIN procedure, model (3.4) was fit to the data as follows: Initial values were selected as 5 for the intercept, 1 for the slope, and 1 for log relative potencies. Derivative statements were included and Gauss-Newton method (default when derivatives are included) was utilized to fit the model. The estimates of log (base 2) of the relative potencies of T_1, T_2, and T_3 and the estimated covariance matrix were found to be identical to those obtained by method 1. The results from the NLIN procedure are given in Appendix B.

5. Discussion

A non-linear reparameterization of the usual parallel line model is a convenient way to approach the problem of combining estimates of relative potency. The NLIN procedure can be used to produce the necessary parameter estimates, and the covariance matrix. An extension of Finney's (1978) method for combining estimates is then easily carried out using the IML procedure code.

6. References

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Stuart, A. & Ord, J.K., Kendall's Advanced Theory of Statistics, Vol 1, Fifth Edition, Oxford University Press, New York, 1987.

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APPENDIX A

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                   PARALLEL LINE  METHOD SECTION
              TABLE III.0.1 - GLM TEST OF PARALLELISM
                  GENERAL LINEAR MODELS PROCEDURE
                      CLASS LEVEL INFORMATION

                 CLASS      LEVELS     VALUES

                 TEST          4       STND T1 T2 T3

              NUMBER OF OBSERVATIONS IN DATA SET = 123

NOTE: ALL DEPENDENT VARIABLES ARE CONSISTENT WITH RESPECT TO THE
      PRESENCE OR ABSENCE OF MISSING VALUES. HOWEVER,
      ONLY    113 OBSERVATIONS CAN BE USED IN THIS ANALYSIS.

DEPENDENT VARIABLE: LPCT

SOURCE                     DF      SUM OF SQUARES         MEAN SQUARE      F VALUE

MODEL                       8       1788.25441179        223.53180147      1182.83
ERROR                     105         19.84294922          0.18898047       PR > F
UNCORRECTED TOTAL         113       1808.09736102                            0.0

R-SQUARE                 C.V.            ROOT MSE           LPCT MEAN

0.989026              11.1058          0.43471884          3.91432402


SOURCE                     DF         TYPE III SS    F VALUE     PR > F

TEST                        4        226.56079042     299.71     0.0001
LDOSE                       1         54.62463262     289.05     0.0001
LDOSE*TEST                  3          0.81473107       1.44     0.2362
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PARALLEL LINE METHOD SECTION

TABLE III.0.4 - REGRESSION ANALYSIS ASSUMING COMMON SLOPE

DEP VARIABLE: LPCT

ANALYSIS OF VARIANCE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PROB>F
MODEL	5	1787.43968	357.48794	1868.975	0.0001
ERROR	108	20.65768029	0.19127482		
U TOTAL	113	1808.09736			

ROOT MSE	0.4373498	R-SQUARE	0.9886
DEP MEAN	3.914324	ADJ R-SQ	0.9880
C.V.	11.17306		

NOTE: NO INTERCEPT TERM IS USED. R-SQUARE IS REDEFINED.

PARAMETER ESTIMATES

VARIABLE	DF	PARAMETER ESTIMATE	STANDARD ERROR	T FOR H0: PARAMETER=0	PROB >]T]
T1	1	7.56034200	0.23196133	32.593	0.0001
T2	1	7.50755685	0.22968943	32.686	0.0001
T3	1	7.43271164	0.23313087	31.882	0.0001
T4	1	7.72601975	0.22987340	33.610	0.0001
LDOSE	1	0.84208782	0.04987580	16.884	0.0001

ESTIMATES OF LOG RELATIVE POTENCY FROM LINEAR REGRESSION MODEL

TEST	ESTIMATED LOG RELATIVE POTENCY
T1	-0.06268
T2	-0.15156
T3	0.19675

ESTIMATED COVARIANCE MATRIX OF LOG RELATIVE POTENCY FROM LINEAR REGRESSION

TEST	T1	T2	T3
T1	0.0193	0.0092966	0.0093085
T2	0.0092966	0.0194	0.0091763
T3	0.0093085	0.0091763	0.0185

APPENDIX B

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NON-LINEAR LEAST SQUARES ITERATIVE PHASE

DEPENDENT VARIABLE: Y METHOD: GAUSS-NEWTON

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
REGRESSION	5	1787.4396807	357.4879361
RESIDUAL	108	20.6576803	0.1912748
UNCORRECTED TOTAL	113	1808.0973610	
(CORRECTED TOTAL)	112	76.7189828	

PARAMETER	ESTIMATE	ASYMPTOTIC STD. ERROR	ASYMPTOTIC 95 % CONFIDENCE INTERVAL LOWER	UPPER
A1	7.56034E+00	2.319613E-01	7.100551E+00	8.020133E+00
B1	8.42088E-01	4.987580E-02	7.432247E-01	9.409510E-01
R2	-6.26837E-02	1.388954E-01	-3.380002E-01	2.126329E-01
R3	-1.51564E-01	1.391940E-01	-4.274727E-01	1.243443E-01
R4	1.96746E-01	1.359418E-01	-7.271570E-02	4.662085E-01

ESTIMATES OF LOG RELATIVE POTENCY FROM NON-LINEAR REGRESSION MODELLING

INTERCEPT FOR STND	COMMON SLOPE	M1	M2	M3
7.56034	0.842088	−0.062684	−0.15156	0.196746

ESTIMATED COVARIANCE MATRIX OF LOG RELATIVE POTENCIES FROM NON-LINEAR REGRESSION MODELLING

TEST	T1	T2	T3
T1	0.0192919	0.0092966	0.0093085
T2	0.0092966	0.0193750	0.0091763
T3	0.0093085	0.0091763	0.0184802